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Heritable Variation in Maternal Yolk Hormone Transfer in a Wild Bird Population

Barbara Tschirren,^{1,*} Joanna Sendecka,² Ton G. G. Groothuis,³ Lars Gustafsson,² and Blandine Doligez⁴

1. Department of Animal Ecology, Lund University, Sölvegatan 37, 22362 Lund, Sweden; 2. Department of Animal Ecology, Uppsala University, Norbyvägen 18, 75236 Uppsala, Sweden; 3. Department of Behavioural Biology, University of Groningen, Kerklaan 30, 9750 AA Haren, The Netherlands; 4. Université de Lyon, Centre National de la Recherche Scientifique, Unité Mixte de Recherche 5558, Laboratoire de Biométrie et Biologie Évolutive, 43 Boulevard du 11 Novembre 1918, Villeurbanne F-69622, France

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ABSTRACT: Differential reproductive investment by the mother can critically influence offspring development and phenotype, and strong selection is therefore expected to act on such maternal effects. Although a genetic basis is a prerequisite for phenotypic traits to respond to selection and thus to evolve, we still know very little about the extent of heritable variation in maternal effects in natural populations. Here, we present the first estimates of intrafemale repeatability across breeding seasons and estimates of heritability of hormone-mediated maternal effects in a wild population of collared flycatchers (*Ficedula albicollis*). We found that maternal yolk testosterone (T) concentrations, yolk mass, and egg mass were moderately to highly repeatable within females across years, whereas intrafemale consistency of maternal yolk androstenedione (A4) deposition was low yet statistically significant. Furthermore, maternal yolk T transfer, yolk mass, and egg mass were significantly heritable, whereas yolk A4 transfer was not. These results strongly suggest that two major maternal yolk androgens are differentially regulated by genes and the environment. Selection on heritable variation in maternal yolk T deposition has the potential to shape the rate and direction of phenotypic change in offspring traits and can thereby accelerate or impede the response to selection in natural populations.

Keywords: egg quality, genetic maternal effects, genetic variation, maternal investment, steroids, transgenerational plasticity.

Introduction

Maternal effects occur when the phenotype of the mother or the environment she encounters modifies her offspring's phenotype (Roach and Wulff 1987; Mousseau and Fox 1998). Such maternal effects are mediated through a diversity of pathways at various reproductive stages, from mate choice until offspring independence (Roach and Wulff 1987; Mousseau and Fox 1998). Maternal effects can

make a significant contribution to variation in fitness by modifying offspring phenotype, thereby altering the genotype-phenotype relationship and accelerating or impeding the response to selection (Kirkpatrick and Lande 1989; Schluter and Gustafsson 1993; Chaverud and Moore 1994; Wolf et al. 1998). They are therefore a very powerful evolutionary force, particularly in species in which females make a considerable investment in their offspring (Reinhold 2002).

In oviparous animals, the embryonic development takes place outside of the mother's body, which makes them excellent models for the study of prenatal maternal effects and their evolutionary consequences. Indeed, maternal investment into the egg (i.e., egg size and composition) has been found to critically influence offspring development in a variety of taxa, including birds, amphibians, reptiles, and fish (e.g., McCormick 1999; Royle et al. 1999; Nager et al. 2000; Lovern and Wade 2001, 2003; Blount et al. 2002; Saino et al. 2002; Räsänen et al. 2005; Warne and Charnov 2008; Dziminski et al. 2009). In particular, androgens of maternal origin, such as testosterone (T) and its precursor androstenedione (A4), which accumulate in the egg yolk during follicle maturation (Schwabl 1993; Schwabl et al. 1997), are an important component of egg quality. They have received much attention over the last few years, as they are thought to be a major determinant of developmental plasticity (e.g., McCormick 1999; Lovern and Wade 2003; Groothuis et al. 2005b; Gil 2008).

Recent work has shown that maternal yolk hormones might be involved in sex determination (Janzen et al. 1998; Bowden et al. 2000; Pike and Petrie 2003) and play an important role in prenatal development (Janzen et al. 1998; Eising et al. 2001). However, also long-lasting organizing effects of maternal yolk hormones on the progeny have been documented, including effects on postnatal growth, begging intensity, and competitiveness (reviewed in Groothuis et al. 2005b and Gil 2008). Moreover, there is accumulating evi-

* Corresponding author; e-mail: barbara.tschirren@zooekol.lu.se.

dence that hormone-mediated maternal effects modulate phenotype and behavior of offspring at adulthood, including aggressiveness and social status (Schwabl 1993; Strasser and Schwabl 2004; Eising et al. 2006), the expression of sexually selected ornaments (Strasser and Schwabl 2004; Eising et al. 2006), the response to novel stimuli (Tobler and Sandell 2007), and natal dispersal behavior (Tschirren et al. 2007).

Whereas the majority of studies have found that maternal yolk androgens are beneficial to offspring (see examples above), elevated levels of yolk androgens may also carry physiological costs for either the mother (Veiga et al. 2004; Veiga and Polo 2008; if high androgen levels in the maternal circulation are required for high androgen levels in the eggs) or the young, potentially through immunosuppressive effects of exposure to androgenic hormones (Groothuis et al. 2005a; Müller et al. 2005; Gil et al. 2006a), resulting in high parasite susceptibility (Tschirren et al. 2004) and a reduced life span, or through higher energy expenditure due to an increased metabolic rate (Tobler et al. 2007a; but see Eising et al. 2003). These opposing costs and benefits indicate that the optimal allocation of maternal yolk androgens into the eggs will depend on current or future environmental or social conditions. Indeed, it is well documented that factors such as breeding density (Schwabl 1997; Reed and Vleck 2001; Groothuis and Schwabl 2002; Mazuc et al. 2003; Pilz and Smith 2004), timing of breeding (Schwabl 1996; Bowden et al. 2000; Pilz et al. 2003; Müller et al. 2004), food abundance (Verboven et al. 2003; Gasparini et al. 2007; Warner et al. 2007), partner attractiveness (Gil et al. 1999, 2004; Tanvez et al. 2004; Loyau et al. 2007; Kingma et al. 2008), or parasitism (Tschirren et al. 2004) influence maternal yolk androgen deposition.

However, despite the fact that maternal plasticity in yolk androgen transfer in response to environmental variation and its adaptive value as a flexible maternal tool to adjust offspring phenotype have received much attention in the recent past, we still know very little about the within-female consistency and the genetic basis of such maternal effects. This study addresses these gaps and presents the first estimates of intrafemale repeatability across breeding seasons and the first heritability estimates of maternal yolk androgen deposition in a wild bird population.

Material and Methods

Study Site, Study Species, and Egg Collection

The study was conducted in 2003 and from 2005 to 2007 in a population of collared flycatchers (*Ficedula albicollis*) breeding in nest boxes on the island of Gotland, Sweden (57°10'N, 18°20'E; Gustafsson 1989; Pärt and Gustafsson

1989; Doligez et al. 2004). At the beginning of the breeding season (from the end of April until the end of May), we regularly visited nest boxes to monitor nest building and egg laying. When two eggs were found in the nest, we marked them with a nontoxic marker. On the next day, we returned to the nest box to collect the third egg on the day it was laid. The collected egg was replaced with a dummy egg to maintain the original clutch size. Collared flycatchers lay one egg per day, and during the study period (2003, 2005–2007) 94% of the females in the population laid a clutch of five to seven eggs. The third egg is thus one of the middle eggs of a clutch. Intraspecific brood parasitism is not observed in the study population (Sheldon and Ellegren 1996).

We weighed the collected eggs (except for two eggs in 2003) to the nearest 0.001 g, separated the yolk from the albumen, weighed the yolk, and froze it at -18°C for later hormone analysis. Mean mass (\pm SD) of collected eggs was $1,763 \pm 138$ mg (range, 1,353–2,206 mg; $N = 344$), and mean yolk mass was 359 ± 32 mg (range, 250–442 mg; $N = 344$). Eggs were collected in 2003 ($N = 79$ analyzed eggs), 2005 ($N = 120$ analyzed eggs), 2006 ($N = 108$ analyzed eggs), and 2007 ($N = 39$ analyzed eggs). No eggs were collected in 2004. The number of eggs collected in 2007 is lower than in the other years because we specifically targeted eggs of previously sampled females and their daughters in the final year of the study.

Females were captured inside the nest box while incubating eggs. We measured their body mass and tarsus length, and if they had not been banded before, they were individually marked with a numbered aluminum ring for identification. Female body condition was calculated as the residuals of body mass on tarsus length. Nestlings were ringed before fledging as part of the long-term monitoring of the study population (Gustafsson 1989; Pärt and Gustafsson 1989; Doligez et al. 2004).

Yolk Androgen Analyses

We analyzed the concentrations of yolk androstenedione (A4) and yolk testosterone (T), the two major androgens of maternal origin in bird eggs (Schwabl 1993; Groothuis et al. 2005b), by radioimmunoassay. The yolks were thawed and homogenized with 400 μL of distilled water. Aliquots of this yolk/water emulsion (approximately 100 mg) were taken, weighed (to the nearest 0.1 mg), and mixed with 150 μL of distilled water and 50 μL of ^3H Tracer T ($\sim 2,000$ counts/min) to assess extraction efficiency. The samples were extracted twice with 2.5 mL of 70% diethyl ether/30% petroleum ether (vol : vol) and dried under a stream of nitrogen. The extracts were then redissolved in 1 mL 70% methanol, centrifuged, and decanted. The supernatant was dried under a stream of nitrogen and redissolved

in phosphate-buffered saline. Testosterone and A4 concentrations were measured in duplicates, using Diagnostic System Laboratories (Webster, TX) radioimmunoassay kits following the manufacturer's protocol.

The average recovery rate was 73% (range, 62%–83%). We corrected measured androgen concentrations (pg/mg yolk) for extraction efficiency. Dilution curves confirmed reliability of extraction and assay protocols. Yolks were randomly distributed across six assays, and we ensured that eggs collected in the same year were analyzed in at least two different assays. We included duplicates of pooled collared flycatcher yolk samples in each assay to calculate intra- and interassay variation. Intra-assay variation was 7.5% for A4 and 6.3% for T. Interassay variation was 5.9% for A4 and 7.7% for T.

Statistical Analyses

We collected eggs of 80 females in two different years and eggs of four females in three different years. Intrafemale repeatability (r) of yolk T and yolk A4 concentrations, egg mass, and yolk mass across years was calculated as $r = s^2_{\text{among females}} / (s^2_{\text{within females}} + s^2_{\text{among females}})$ (Lessells and Boag 1987). Standard errors were calculated following Becker (1984). In addition, we calculated the repeatability of egg size and egg composition corrected for variation among years using residual values from an ANOVA with year as a random effect.

Heritability (h^2) of maternal yolk androgen (T and A4) concentrations, egg mass, and yolk mass was calculated as twice the slope (b) of the corresponding mother-daughter regression (Lynch and Walsh 1998). The standard error (SE) of h^2 was calculated as twice the SE of b (Lynch and Walsh 1998). Eggs of 57 mother-daughter pairs were collected. Mean values were used if a female was sampled in more than 1 year. For five mothers, we collected eggs of two daughters. We used mean daughter values for these mothers to ensure that each mother ($N = 52$) was included only once in the analysis. We also calculated the heritability of egg size and composition corrected for female body condition, using the residuals of a regression of egg traits on female body condition in the mother-daughter regression. Sample sizes are smaller for these analyses because some females were not measured. Some of the sampled daughters ($N = 14$) were cross-fostered shortly after hatching and were thus not raised by their biological parents. We repeated the mother-daughter regressions using this subsample of cross-fostered daughters only. We calculated Pearson's correlation coefficients to estimate the relationship between different egg components and the relationship between egg components and female body condition. We checked the residuals of all models for normality, using Shapiro-Wilks tests (all $P >$

.206), and used the software package JMP 5 (SAS Institute 1989–2002) for the statistical analyses.

Results

Correlates of Egg Size and Composition

Mean yolk hormone concentration (\pm SD) was 211.6 ± 60.4 pg/mg yolk (range, 82–425 pg/mg yolk) for A4 and 39.9 ± 14.2 pg/mg yolk (range, 18–138 pg/mg yolk) for T. Yolk A4 and yolk T concentrations were not significantly related to egg mass (A4, $r = 0.041$, $P = .448$; T, $r = 0.050$, $P = .350$; $N = 344$) or yolk mass (A4, $r = 0.042$, $P = .443$; T, $r = -0.017$, $P = .755$; $N = 344$). The A4 and T concentrations were positively correlated within eggs ($r = 0.395$, $P < .001$; $N = 346$). Similarly, egg mass and yolk mass were positively correlated ($r = 0.544$, $P < .001$; $N = 344$).

Female body condition was significantly positively associated with egg mass ($r = 0.221$, $P < .001$; $N = 333$) and yolk mass ($r = 0.133$, $P = .015$; $N = 333$), significantly negatively associated with yolk A4 ($r = -0.124$, $P = .024$; $N = 335$), and not significantly associated with yolk T ($r = 0.066$, $P = .229$; $N = 335$). In females that have been sampled in more than 1 year, there was no relationship between the change in body condition and the change in yolk T ($r = 0.108$, $P = .340$; $N = 80$), yolk A4 ($r = -0.171$, $P = .130$; $N = 80$), egg mass ($r = 0.104$, $P = .360$; $N = 80$), or yolk mass ($r = -0.055$, $P = .629$; $N = 80$). The average time gap (\pm SD) between sampling of the first and second (or third) egg of the same female in subsequent years was 1.5 ± 0.9 years (range, 1–4). We found no significant relationship between the length of the gap and the change in yolk T, yolk A4, egg mass, or yolk mass (repeated-measures ANOVA; all $P > .407$).

Repeatability of Egg Size and Composition

Yolk androgen concentrations, egg mass, and yolk mass were all repeatable within females across breeding seasons (table 1). Yolk A4 differed significantly among years ($F = 31.024$, $df = 3, 85$, $P < .001$), with levels in 2005 be-

Table 1: Repeatability of egg mass and composition

Maternal effect	$r \pm 1 \text{ SE}$	F	df	P
Yolk T	.596 \pm .069	4.018	83, 88	<.001
Yolk A4	.217 \pm .102	1.567	83, 88	.019
Egg mass	.706 \pm .054	5.926	83, 88	<.001
Yolk mass	.477 \pm .083	2.869	83, 88	<.001

Note: Intrafemale repeatability (r) of yolk testosterone (T) concentration, yolk androstenedione (A4) concentration, egg mass, and yolk mass across breeding seasons. Eighty females have been sampled in two different years, and four females have been sampled in three different years.

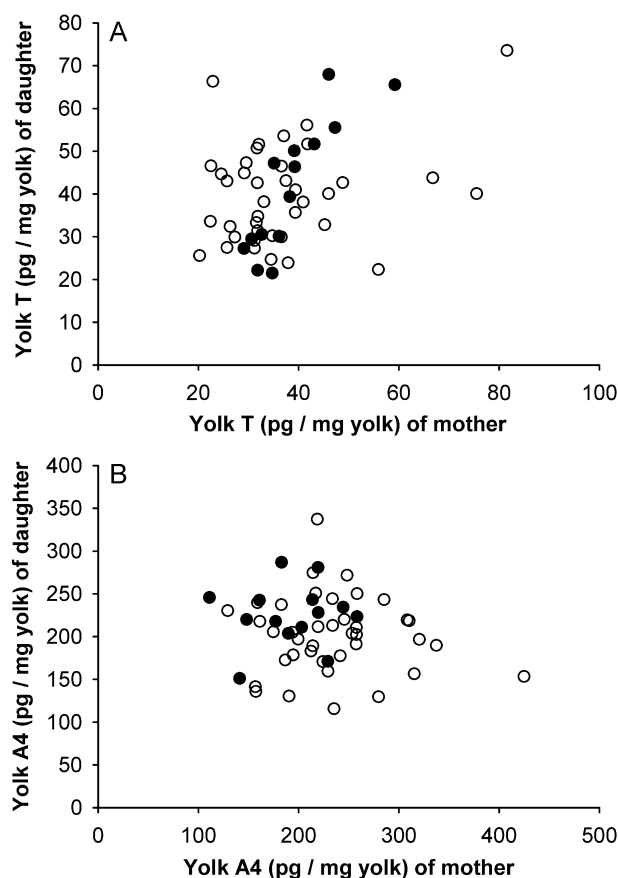


Figure 1: Comparison of yolk androgen concentrations in the eggs of mothers and daughters. A, Yolk testosterone (T); and B, yolk androstenedione (A4) concentrations. Filled circles represent daughters raised by foster parents ($N = 14$); open circles represent daughters raised by their biological parents ($N = 43$). The mother-daughter resemblance was significant for yolk T, both overall and when considering only cross-fostered daughters. No significant relationship between mothers and daughters was observed for yolk A4.

ing significantly lower than in other years (contrast 2005 vs. other years: $F = 72.022$, $df = 1, 85$, $P < .001$). Similarly, there was a significant year effect on yolk mass ($F = 4.069$, $df = 3, 85$, $P = .009$), with yolks in 2003 being significantly heavier than in other years (contrast 2003 vs. other years: $F = 9.258$, $df = 1, 85$, $P = .003$). No significant year effect on yolk T ($F = 0.649$, $df = 3, 85$, $P = .586$) or egg mass ($F = 0.718$, $df = 3, 85$, $P = .544$) was observed. When accounting for year effects, repeatability was 0.455 ± 0.085 for yolk A4, 0.596 ± 0.069 for yolk T, 0.487 ± 0.082 for yolk mass, and 0.703 ± 0.055 for egg mass (all $P < .001$).

Heritability of Egg Size and Composition

Egg composition of daughters resembled that of their mothers, with yolk T concentration (slope ± 1 SE; $b =$

0.373 ± 0.131 ; fig. 1A), egg mass ($b = 0.421 \pm 0.151$), and yolk mass ($b = 0.484 \pm 0.163$) all being significantly heritable (table 2). Yolk A4 concentrations of mothers and daughters, however, were unrelated ($b = -0.099 \pm 0.107$; table 2; fig. 1B), and yolk A4 transfer remained nonheritable, even after accounting for significant among-year variation ($b = -0.094 \pm 0.108$; $F = 0.753$, $df = 1, 50$, $P = .390$). The heritability estimates of yolk T and yolk A4 are significantly different because their 95% confidence intervals do not overlap (table 2).

The analysis of residual egg composition (accounting for female body condition) gave similar heritability estimates (yolk T: $h^2 = 0.70 \pm 0.25$; $F = 7.634$, $df = 1, 41$, $P = .009$; yolk A4: $h^2 = -0.28 \pm 0.23$; $F = 1.565$, $df = 1, 41$, $P = .218$; egg mass: $h^2 = 0.75 \pm 0.36$; $F = 4.348$, $df = 1, 41$, $P = .043$; yolk mass: $h^2 = 1.29 \pm 0.30$; $F = 17.944$, $df = 1, 41$, $P < .001$). In the subsample of daughters that had been raised by foster parents, we observed a significant biological mother-daughter resemblance in yolk T transfer ($F = 32.771$, $df = 1, 12$, $P < .001$; fig. 1A) but not in yolk A4 transfer ($F = 0.073$, $df = 1, 12$, $P = .791$; fig. 1B), egg mass ($F = 0.131$, $df = 1, 12$, $P = .724$), or yolk mass ($F = 1.128$, $df = 1, 12$, $P = .309$).

Discussion

Despite the increasing number of studies demonstrating the important role of yolk androgens in transgenerational developmental plasticity in various taxa, we still know very little about the physiological mechanisms and the genetic basis underlying such maternal effects (Mousseau and Fox 1998; Gil 2008). The latter is of particular interest because genetic maternal effects are a heritable component of the environment provided by the mother that itself can evolve (Mousseau and Fox 1998; Wolf et al. 1998; Wolf 2003; see also Shaw and Byers 1998 for a review of genetic maternal effects in plants).

So far, very few studies (table 3) have estimated the within-female consistency of yolk hormone transfer, which can be considered an upper-bound estimate of its heritability (Lynch and Walsh 1998). We observed a high ($r = 0.60$) within-female repeatability of yolk T deposi-

Table 2: Heritability of egg mass and composition

Maternal effect	h^2	95% CI	F	df	P
Yolk T	.746	.23 to 1.26	8.088	1, 50	.006
Yolk A4	-.197	-.62 to .22	.844	1, 50	.363
Egg mass	.842	.25 to 1.43	7.764	1, 50	.008
Yolk mass	.968	.33 to 1.61	8.877	1, 50	.004

Note: Heritability estimates (h^2) and 95% confidence intervals (CIs) of yolk testosterone (T) concentration, yolk androstenedione (A4) concentration, egg mass, and yolk mass; $N = 52$ mother-daughter pairs.

Table 3: Published repeatability estimates of maternal yolk hormone transfer

Species/yolk hormone	<i>r</i>	<i>N</i>	Sampling interval	Reference
<i>Ficedula hypoleuca</i> (wild):				
Yolk T	.56	16	<2 weeks	Tobler et al. 2007 <i>b</i>
Yolk A4	.58	16	<2 weeks	Tobler et al. 2007 <i>b</i>
<i>Hirundo rustica</i> (wild):				
Yolk A4	.22	64	>4 weeks	Gil et al. 2006 <i>b</i>
<i>Sturnus vulgaris</i> (captive):				
Yolk T	.50	12	1 year	Eising et al. 2008
Yolk A4	.43	14	1 year	Eising et al. 2008

Note: T, testosterone; A4, androstenedione. Sampling interval indicates the time between egg collections.

tion across years, which is in accordance with previous work that estimated within-female consistency of yolk androgen transfer over a shorter time span or in captivity (table 3). The repeatability of yolk A4 transfer across breeding seasons, however, was low ($r = 0.22$; see also Gil et al. 2006*b*), which is indicative of a high environmental sensitivity of this maternal trait. Indeed, we found significant year effects on yolk A4 concentrations as well as a significant negative association between female body condition and yolk A4. No such effects were observed for yolk T. Furthermore, experimental manipulation of environmental conditions (e.g., parasitism [Tschirren et al. 2004], food abundance [Verboven et al. 2003], and immune challenge of the mother [Gil et al. 2006*a*]) had stronger effects on the yolk A4 than on the yolk T content of eggs (but see Reed and Vleck 2001; Groothuis and Schwabl 2002; Pilz and Smith 2004). The finding that repeatability of A4 was substantially higher when correcting for variation among years indicates that although females deposit variable amounts of A4 into the eggs depending on environmental conditions, they deposit similar amounts relative to each other, showing an interesting interaction between plasticity on the one hand and individual consistency on the other.

Consistent with the differences in intrafemale repeatability across breeding seasons, we observed differences in the mother-daughter resemblance of yolk T and yolk A4 transfer. To put the heritability estimates of yolk androgen deposition into perspective, we estimated the heritability of egg mass and yolk mass, which are considered to be highly heritable maternal traits (reviewed in Christians 2002). The heritability of yolk T ($h^2 = 0.75$) was high and comparable to the heritability of egg mass ($h^2 = 0.84$) and yolk mass ($h^2 = 0.97$) observed in our study as well as in other species (Christians 2002). This finding adds to the evidence for a genetic basis of prenatal maternal effects, which is well established for livestock (e.g., Meyer 1997; Dodenhoff et al. 1999; Moce et al. 2004) and plants (reviewed in Shaw and Byers 1998) but still scarce for free-

living animals. Yolk A4 deposition, on the other hand, was not heritable. These contrasting heritability patterns are in accordance with the findings of a selection experiment in Japanese quails (*Coturnix coturnix japonica*) in which artificial selection for divergent social behavior resulted in a correlated response in yolk T levels but not in yolk A4 (Gil and Faure 2007), corroborating our finding that yolk A4 and yolk T are differently influenced by genes and the environment. However, selection for exploration behavior in great tits (*Parus major*) resulted in an equally strong effect on both androgens (Groothuis et al. 2008).

Measuring heritability in a nonexperimental setup can be problematic because offspring might tend to experience similar environmental conditions as do their parents, thus inflating the heritability estimates of measured traits (Merilä and Sheldon 2000). Indeed, egg mass and yolk mass were no longer heritable when considering only females that had been cross-fostered shortly after birth. Although sample sizes of cross-fostered females are small, it suggests that common environmental effects might at least partly contribute to the often observed mother-daughter resemblance in egg mass (reviewed in Christians 2002) and that the heritability of egg mass might thus in some cases be overestimated.

For yolk T deposition, however, a significant resemblance between daughters and their biological mothers was also found in the subsample of cross-fostered birds. This suggests that common environmental effects are less likely as an explanation for the significant heritability of maternal T transfer. However, we cannot exclude the interesting alternative that early maternal effects (especially the yolk T content of an egg) determine the daughters' deposition of yolk T later in life. Such a maternal priming effect could lead to a strong mother-daughter resemblance in yolk T deposition as observed in our study. Prenatal exposure to androgens is known to have organizing effects on adult physiology, and exposure to elevated levels of maternal T might induce a transgenerational effect by influencing the hypothalamic-pituitary-gonadal axis. Indeed, intergener-

ational effects by DNA methylation of genes coding for hormone receptors are well known in rats (Weaver et al. 2004; Champagne et al. 2006). Only an experimental manipulation of yolk T and the subsequent analysis of the yolk T content of the daughters' eggs will allow us to distinguish between a genetic determination of yolk T deposition and a maternal priming effect. Importantly, however, both pathways will lead to consistent variation in maternal yolk T deposition among families and thus have similar evolutionary implications.

In conclusion, we present direct evidence for consistent, heritable variation in maternal yolk testosterone transfer in a wild bird population and clear differences in the repeatability and heritability of different hormonal components of an egg. Importantly, a high consistency of yolk T deposition within females and between mothers and daughters does not preclude a plastic and adaptive female response to environmental variation, but in addition, it opens up the opportunity for hormone-mediated maternal effects to respond to selection and thus to evolve. Such indirect genetic effects can modify or accelerate phenotypic change in natural populations and might thereby play an important role in evolutionary processes such as parent-offspring coevolution (Kölliker et al. 2000, 2005; Müller et al. 2007; Tschirren and Richner 2008), the evolution of behavioral syndromes (Dingemanse et al. 2003; van Oers et al. 2004; Groothuis and Carere 2005; Gil and Faure 2007; Tobler and Sandell 2007; Groothuis et al. 2008), or dispersal behavior and the colonization of new environments (Hahn et al. 2005; Duckworth and Badyaev 2007; Tschirren et al. 2007) in a range of taxa. We hope that by elucidating the heritable basis of yolk hormone deposition, this study will bring us closer to an understanding of the evolution of hormone-mediated maternal effects, the patterns that shape their current expression both within and among species, and their consequences for the direction and speed of phenotypic change in natural populations.

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